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Introduction

The EZgene™ family of products is an innovative system that radically simplifies the extraction and purification of nucleic acids from a variety of sources. The key to this system is the new ezBind™ matrix that specifically, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or a low salt buffer.

The EZgene™ Tissue DNA Kit provides an easy and rapid method for the isolation of genomic DNA for consistent PCR and Southern analysis. Up to 30 mg of animal tissue, culture cells, mouse tail snips and paraffin-embedded tissue can be readily processed at a time. This kit allows for the single or multiple simultaneous processing of samples. There is no need for phenol/chloroform extractions, and time-consuming steps are eliminated (e.g. precipitation using isopropanol or ethanol). Purified DNA can be directly used for most applications such as PCR, Southern Blotting, and Restriction Enzyme Digestion.

Storage and Stability

All EZgene™ Tissue DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: reconstituted Proteinase K -20 °C, and all other materials at RT (22-25 °C).

Binding Capacity

Each ezBind™ DNA mini column can bind approximately **100 µg DNA**. Using greater than **30 mg tissue** or **1x10⁷ cells** is not recommended.

Kit Contents

Catalog#	GD2211-00	GD2211-01	GD2211-02
Preps	4	50	250
ezBind DNA Mini Columns	4	50	250
collection tube	8	100	500
Buffer TL	1.2 mL	15 mL	70 mL
Buffer BL	1.2 mL	15 mL	70 mL
Buffer KB	2.4 mL	28 mL	135 mL
DNA Wash Buffer**	2 mL	15 mL	3 x 24 mL
Elution Buffer*	2 mL	15 mL	70 mL
Proteinase K	3 mg	30 mg	5 x 30 mg
User Manual	1	1	1

*Elution Buffer = 10 mM Tris-HCl, pH 8.5

****Add 8 mL (GD2211-00) or 60 mL (GD2211-01) or 96 mL (GD2211-02) absolute ethanol (96-100%) to each DNA Wash Buffer bottle before use.**

Safety Information

Buffer BL contains Chaotropic salts. Use gloves and protective eyewear when handling this solution.

Before Starting

EZgene™ Kits are designed to be simple, fast, and reliable provided that all steps are followed diligently. It is strongly advised that you familiarize yourself with the entire booklet before starting.

Important Notes

- ☒ Reconstitute each vial of Protease K 110 µL (GD2211-00), 1.3 mL (GD2211-01), or 5 x 1.3 mL (GD2211-02) of Elution Buffer. Vortex the vial briefly before use. We recommend that you aliquot, and store vials of Proteinase K at -20 °C. Bring Proteinase K solution to room temperature before use.
- ☒ **Dilute DNA Wash Buffer with absolute ethanol as follows:**
Add 8 mL (GD2211-00) or 60 mL (GD2211-01) or 96 mL (GD2211-02) absolute ethanol (96-100%) to each bottle.
- ☒ **Preheat Elution Buffer to 70 °C (in aliquots of 0.5 mL/ sample).**
- ☒ **For Mouse Tail Protocol have the following ready:** Pre-mixed solutions of 220 µL of Buffer BL and 220 µL of absolute ethanol (per sample). Mix by vortexing. They can be prepared fresh, or pre-made and stored at room temperature less than one month.

Materials and Reagents to be supplied by user

- ☒ Tabletop microcentrifuge.
- ☒ Sterile 1.5 mL centrifuge tubes.
- ☒ Shaking water-bath set to 55 °C for Tissue Protocol; 65 °C for Cultured Cells; 55 °C for Mouse Tail.
- ☒ RNase Stock Solution (100 mg/mL) (**OPTIONAL**).
- ☒ Absolute Ethanol (96-100%).
- ☒ PBS Buffer.

Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl Buffer, or Elution Buffer as a blank. DNA concentration is calculated as:

$$[\text{DNA}] = (\text{Absorbance}_{260}) \times (0.05 \text{ } \mu\text{g}/\mu\text{L}) \times (\text{Dilution Factor})$$

The quality of DNA can be assessed by measuring absorbance at both 260 nm and 280 nm. An A_{260}/A_{280} ratio of 1.7-1.9 corresponds to 85%-95% purity.

Expected yields vary with both amount and type of tissue used.

Typically, 30 mg of fresh tissue will yield 10~40 μg of DNA with two elutions (each 200 μL).

Concentrate DNA

If necessary, the DNA can be concentrated as follows. Add sodium chloride to reach a final concentration of 0.1M followed by 2 x volume of absolute (96-100%) ethanol. Mix well and incubate at -20 °C for 10 min. Centrifuge at 10,000 x g for 15 min and discard supernatant. Add 700 μL of 70% ethanol and centrifuge at 10,000 x g for 2 min. Discard supernatant, air dry the pellet for 2 min, and resuspend the DNA in 20 μL of sterile deionized water or 10 mM Tris-HCl, pH 8.5.

A. EZgene™ Tissue Genomic DNA Miniprep Protocol

This method is suitable for the isolation of genomic DNA from up to 30 mg of tissue. Yields vary depending on source.

Optional: Although no mechanical homogenization of tissue is necessary, pulverizing the samples in liquid nitrogen will improve lysis and reduce incubation time. Once the liquid nitrogen has evaporated, transfer the powdered tissue into a clean 1.5 mL tube. Add 200 µL of Buffer TL and proceed to step 2 below.

1. Mince up to **20-30 mg tissue** and place it into a 1.5 mL microtube. Add **200 µL Buffer TL**. In order to speed up lysis, cut the tissue into small pieces. For samples more than 30 mg, simply scale up the volume of Buffer TL used. For example, **400 µL of Buffer TL** should be used for 40-60 mg sample.
2. Add **25 µL reconstituted Proteinase K**. Vortex to mix, and incubate in a shaking water bath set to 55 °C to effectively complete lysis. If no shaking water-bath is available, vortex the sample every 20-30 min.

Note: Lysis time depends on the amount and type of tissue used; average time is usually under 3 hours. One can allow lysis to proceed overnight.

3. **Optional:** Certain tissues such as liver tissue have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. To do so, add **4 µL** (assuming a sample size of 30 mg) **RNase A** (20 mg/mL) and incubate at room temperature for 2 min. Proceed with the tissue protocol.
4. Centrifuge for 5 min at $\geq 13,000 \times g$ to pellet insoluble tissue debris. CAREFULLY aspirate the supernatant and transfer to a sterile microtube.
5. Add **220 µL Buffer BL**. Vortex to mix. Incubate at 70 °C for 10 min.

Note: A wispy precipitate may form upon addition of Buffer BL, but does not interfere with DNA recovery. Adjust the volume of Buffer BL required based on the amount of starting material.

6. Add **220 µL Absolute Ethanol (96-100%, room temperature)**. Vortex to mix.

Note: Adjust the volume of ethanol required based on the amount of starting material.

7. Insert an ezBind™ DNA Mini Column into a 2 mL collection tube. Transfer the entire sample from step 6 into the column including any precipitate that may have formed. Centrifuge at 10,000 x g for 1 min. Discard the collection tube with flow-through liquid.
8. Place the DNA Mini Column into a new 2 mL collection tube and add **500 µL Buffer KB**. Centrifuge at 10,000 x g for 1 min. Discard collection tube and flow-through liquid.
9. Place the DNA Mini Column into a new 2 mL collection tube. Add **650 µL DNA Wash Buffer**. Centrifuge as above and discard flow-through liquid.

Note: Ensure that absolute ethanol has been added to the DNA Wash Buffer before use. See label for directions. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.

10. Using the same 2 mL collection tube, Add **650 µL DNA Wash Buffer**. *Repeat step 9.*
11. Using the same 2 mL collection tube, and centrifuge with the lid open at maximum speed ($\geq 13,000 \times g$) for 2 min to remove the residual ethanol.

Note: This step is crucial for ensuring optimal elution.

12. Place the DNA Mini Column into a sterile 1.5 mL microtube, and add **100-200 µL preheated (70 °C) Elution Buffer (10 mM Tris, pH8.5)**. Allow to sit at room temperature for 1-3 min. Centrifuge at $\geq 13,000 \times g$ for 1 min.
13. **Optional: Repeat the elution with a second 100-200 µL Elution Buffer.**

Note: Each **200 µL** elution typically produces yields of 60-70% of the DNA bound to the column. Thus two elutions will generally give ~90% yields. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using **50-100 µL** of **Elution Buffer** (which slightly reduces overall DNA yield). Volumes lower than 50 µL greatly reduce yields. In some instances,

yields may be increased by incubating the column at 70 °C (rather than at room temperature) upon the addition of Elution Buffer.

B. EZgene™ Cultured Cell Genomic DNA Miniprep Protocol

This protocol is designed for the rapid isolation of up to 25 µg of genomic DNA from up to 5×10^6 cultured cells.

1. Prepare the cell suspension

- a. Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation. Wash the cells with PBS, and resuspend cells with **200 µL Buffer TL**.
 - b. For cells grown in suspension, pellet 5×10^6 cells by spinning at $1,200 \times g$ in a centrifuge tube. Discard the supernatant, and wash the cells once with PBS. Resuspend cells with **200 µL Buffer TL**.
 - c. For cells grown in a monolayer, harvest the cell by scraping with a rubber policeman. Wash cells twice, and resuspend cells with **200 µL Buffer TL**.
2. Add **25 µL reconstituted Proteinase K**. Vortex to mix well, and incubate in a 65 °C shaking water-bath to complete lysis effectively.
3. **OPTIONAL:** Cultured cells have high levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. To do so, add **4 µL of RNase A (100 mg/mL)** and incubate at room temperature for 2 minutes.
4. Add **220 µL Buffer BL** and vortex to mix. Incubate at 70 °C for 10 min.

Note: A wispy precipitate may form upon addition of Buffer BL, but does not interfere with DNA recovery.

5. Add **220 µL Absolute Ethanol (96-100%, room temperature)** and mix thoroughly by vortexing.
6. Insert an ezBind™ DNA Mini Column into a 2 mL collection tube. Transfer the entire sample from step 5 into the column including any precipitate that may have formed. Centrifuge at $10,000 \times g$ for 1 min to bind DNA. Discard the collection tube with flow-through liquid.

7. Place the Mini Column into a new collection tube. Add **500 µL Buffer KB**. Centrifuge at 10,000 x g for 1 min. Discard collection tube and flow-through liquid once again.
8. Place the Mini Column into a new collection tube. Add **650 µL DNA Wash Buffer** diluted with absolute ethanol. Centrifuge as above and discard flow-through liquid.

Note: Ensure that ethanol has been added to DNA Wash Buffer. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.

9. Using the same 2 mL collection tube. Add **650 µL DNA Wash Buffer**, repeat Step 8.
10. Using the same 2 mL collection tube, centrifuge at a maximum speed (13,000 x g) for 2 min to remove the residual ethanol.

Note: This step is crucial for ensuring optimal elution.

11. Place the ezBind DNA Mini Column into a sterile 1.5 mL microtube. Add **100-200 µL preheated (70 °C) Elution Buffer**. Allow to sit at room temperature for 3 minutes. Centrifuge at 13,000 x g for 1 min.
12. **Optional: Repeat the elution with a second 100-200 µL Elution Buffer.**

Note: Each **200 µL** elution will typically produces yields of 60-70% of the DNA bound to the column. Thus two elutions will generally give ~90% yields. However, increasing the elution volume will reduce the concentration of the final product. To obtain DNA at higher concentrations, elution can be carried out using **50 µL -100 µL of Elution Buffer** (which slightly reduces overall DNA yield). Volumes lower than **50 µL** greatly reduce yields. In some instances yields may be increased by incubating the column at 70 °C (rather than at room temperature) upon the addition of Elution Buffer.

C. EZgene™ Mouse Tail Genomic DNA Miniprep Protocol

Bring frozen samples and Proteinase K solution to room temperature, and pre-heat aliquots of Elution Buffer (0.5 mL / sample) at 70 °C.

1. Snip two pieces of mouse tail **0.2 - 0.5 cm** in length. Place into a sterile 1.5 mL microcentrifuge tube, and add **180 µL Buffer TL**.

Note: If necessary cauterize the wound to stop bleeding. Having appropriately earmarked the animal, return it to a clean cage. Mice should not be older than 6 weeks, since lysis will be more difficult which results in suboptimal DNA yields. If possible, obtain a tail biopsy at 2-4 weeks and freeze samples at -70 °C until DNA is extracted.

2. Add **25 µL reconstituted Proteinase K**. Vortex to mix well, and incubate in a shaking water bath set to 55 °C for 1-4 hours or until lysis is complete. If no shaking water-bath is available, vortex the sample every 20-30 min.

Note: Incomplete lysis may block column flow and significantly reduce DNA yields. Incubation time for complete tail lysis is dependent on tail length, and animal age; 0.5 cm tail pieces from a 2-week old mice typically lyses in approximately 2 hours. For older animals an overnight incubation may improve yields. Bone and hair will not be lysed.

3. **OPTIONAL:** mouse tail snips have low levels of RNA which will be co-purified with DNA using this kit. While it will not interfere with PCR, the RNA may be removed at this point. To do so, add **4 µL of RNase A** (100 mg/mL) and incubate at room temperature for 2 minutes. Proceed with the following protocol.
4. Centrifuge for 5 min at 12,000 x g to pellet insoluble tissue debris and hair. CAREFULLY aspirate the supernatant, and transfer to a sterile 1.5 mL microtube, while leaving behind any insoluble pellet.
5. Add **1 volume Buffer BL** followed by **1 volume Absolute Ethanol**.

Alternatively, user may add **2 volumes of premixed BL/ethanol mixture** to the sample. Vortex thoroughly to mix. Thorough mixing is essential at this point.

6. Insert an ezBind™ DNA Mini Column into a 2 mL collection tube. Transfer the entire sample from Step 5 into the column including any wispy precipitate that may have formed.
7. Centrifuge at 10,000 x g for 1 min to bind DNA. Discard the collection tube with through liquid.
8. Place the Mini Column into a new collection tube. Add **500 µL Buffer KB**. Centrifuge at 10,000 x g for 1 min. Discard collection tube the flow-through liquid.
9. Place the Mini Column into a new collection tube. Add **650 µL DNA Wash Buffer**. Centrifuge at 10,000 x g for 1 min. Discard flow-through liquid

Note: Ensure that absolute ethanol has been added to the DNA Wash Buffer before use. If refrigerated, DNA Wash Buffer must be brought to room temperature before use.
10. Using the same 2 mL collection tube, Add **650 µL DNA Wash Buffer**, repeat step 9.
11. Using the same 2 mL collection tube, centrifuge with the lid open at maximum speed of 13,000 x g for 2 min to remove residual ethanol.

Note: This step is crucial for ensuring optimal elution in the following step.
12. Place the Mini Column into a sterile 1.5 mL microtube, and add **100-200 µL of preheated (70 °C) Elution Buffer**. Allow to sit at room temperature for 3 minutes. Centrifuge at 13,000 x g for 1 min.
13. **Optional: Repeat the elution with a second 100-200 µL Elution Buffer.**

D. EZgene™ Paraffin-Embedded Tissue Genomic DNA Miniprep Protocol

1. Place no more than **30 mg** of tissue ($\sim 2 \text{ mm}^3$) in a clean 2 mL microtube.
2. Extract the sample with **1 mL xylene (not supplied)** to remove the paraffin. Mix thoroughly by vortexing.
3. Centrifuge the tube at 10,000 x g at room temperature for 10 min. Discard the supernatant without disturbing the pellet.
4. Rinse the pellet with **1 mL Absolute Ethanol** to remove any traces of xylene. Centrifuge at 10,000 x g for 5 min at room temperature. Discard the ethanol without disturbing the tissue pellet.
5. REPEAT the ethanol rinse.
6. Air dry the tissue pellet at 37 °C for 15 min.
7. Add **200 µL Buffer TL** to the tissue and then follow **the standard Tissue DNA Isolation Protocol on page 6**, starting with step 2 (using reconstituted Proteinase K).

For DNA elution, we recommend using **50-100 µL of Elution Buffer** warmed to 70 °C. Yields will depend on size and age of sample. Certain samples may require prolonged lysis with Buffer TL.

Tissue fixed with paraformaldehyde will yield degraded DNA or RNA. The extent of degradation depends on type of fixative used, but the size of DNA obtained is usually less than 500 bp. Degradation is not caused by the EZgene™ Tissue DNA Protocol. For PCR detection of segments smaller than 500 bp, satisfactory results can be obtained.

EZgene™ Vacuum/Spin Protocol

Carry out disruption, homogenization, protease digestion, and loading onto the ezBind™ DNA Mini Column as indicated in previous protocols. Instead of continuing with centrifugation, follow the steps outlined below.

Note: Please read through previous sections of this book before beginning this protocol.

1. Prepare the vacuum manifold according to manufacturer's instruction, and connect the ezBind™ DNA Spin Column to the manifold.
2. Load the sample onto the column.
3. Switch on vacuum to draw the sample through the column, and then turn off the vacuum.
4. Wash the column by adding **500 µL Buffer KB**. Draw the liquid through the column by turning on the vacuum.
5. Wash the column by adding **650 µL DNA Wash Buffer**. Draw the Wash Buffer through the column by turning on the vacuum source.
6. Washing the column with another **650 µL DNA Wash Buffer**.
7. Place the column into a 2 mL collection tube, and transfer the column into a microcentrifuge. Spin for 2 min at 13,000 x g with the lid open to dry the column.
8. Place the column into a clean 1.5 mL microtube, and add **100-200 µL of preheated (70 °C) Elution Buffer**. Let it sit at room temperature for 1-2 min, and then centrifuge at 13,000 x g for 1 min to elute DNA.
9. Place the Mini Column into a sterile 1.5 mL microtube, and add **100-200 µL of preheated (70 °C) Elution Buffer**. Allow to sit at room temperature for 3 minutes. Centrifuge at 13,000 x g for 1 min.
10. **Optional:** Repeat the elution with a second **100-200 µL Elution Buffer**.

Trouble Shooting Guide

Problem	Possible reason	Suggested improvement
Clogged Column	Incomplete Lysis	Extend incubation time of lysis with Buffer TL, and Proteinase K. Add the correct volume of Buffer BL and incubate for specified time at 70 °C. It may be necessary to extend incubation time by 10 min.
	Sample is too Large	If using more than 30 mg tissue, increase volumes of Proteinase K, Buffer TL, Buffer BL, and ethanol. Pass aliquots of lysate through one column successively.
	Sample is too viscous	Divide sample into multiple tubes, and adjust the volume to 25 µL with Buffer TL.
Low DNA Yield	Clogged Column	See above
	Poor elution	Repeat elution or increase elution volume (see notes on elution, at the end of each protocol). Incubation of column at 70 °C for 5 min with Elution Buffer may increase yields.
	Improper washing	DNA Wash Buffer must be diluted with absolute (96-100%) ethanol as specified on page 4 before use.
	Culture overgrown	Overgrown culture contains lysed cells and degraded DNA. Do not grow cell for longer than 16 hours.
	Sample has low DNA content	Increase starting material and volume of all reagents (Proteinase K, Buffer TL, Buffer BL, and ethanol) proportionally. Pass aliquots of lysate through column successively.
Low A_{260}/A_{280} ratio	Extended centrifugation during elution	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation-it will not interfere with PCR or restriction digests.
	Poor cell lysis due to incomplete mixing with Buffer BL	Repeat the procedure, this time making sure to vortex the sample with Buffer BL immediately and completely.
	Incomplete cell lysis or protein degradation due to insufficient incubation	Increase incubation time with Buffer TL, and Proteinase K. Ensure that no visible pieces of tissue remain.

Related EZgene™ Products

Catalog #	Product Name	Preps	Price \$
GD2211-01	Tissue gDNA kit	50	90.00
GD2211-02	Tissue gDNA kit	250	420.00
GD2814-01	96-well tissue gDNA kit	4x96	680.00
GD2814-02	96-well tissue gDNA kit	20x96	3000.00
GD2311-01	Blood gDNA mini kit	50	90.00
GD2311-02	Blood gDNA mini kit	250	420.00
GD2312-01	Blood gDNA midi kit	10	90.00
GD2312-02	Blood gDNA midi kit	25	200.00
GD2314-01	Blood gDNA maxi kit	10	160.00
GD2314-02	Blood gDNA maxi kit	25	360.00
GD2815-01	96-well blood gDNA kit	4x96	680.00
GD2815-02	96-well blood gDNA kit	20x96	3000.00
GD2411-01	Bacterial gDNA kit	50	110.00
GD2411-02	Bacterial gDNA kit	250	495.00
GD2412-01	Soil gDNA kit	50	90.00
GD2412-02	Soil gDNA kit	250	420.00
GD2413-01	Insect gDNA kit	50	90.00
GD2413-02	Insect gDNA kit	250	420.00
GD2415-01	Yeast gDNA kit	50	90.00
GD2415-02	Yeast gDNA kit	250	420.00
GD2416-01	Fungal gDNA kit	50	90.00
GD2416-02	Fungal gDNA kit	250	420.00